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# $\beta_3$-Adrenoceptor Is the Predominant $\beta$-Adrenoceptor Subtype in Human Myometrium and Its Expression Is Up-Regulated in Pregnancy

C. Rouget, M. Bardou, M. Breuiller-Fouché, C. Loustalot, H. Qi, E. Naline, T. Croci, D. Cabrol, C. Advenier, and M. J. Leroy

*Unité Propre de Recherche de l'Enseignement Supérieur EA220–Pharmacology (C.R., M.B., H.Q., E.N., C.A.), Unité de Formation et de Recherche Biomédicale des Saints Pères, 75006 Paris, France; Institut National de la Santé et de la Recherche Médicale U427 (C.R., M.B.-F., H.Q., D.C., M.J.L.) and Department of Gynecology (D.C.), Port-Royal Maternity Hospital, 75014 Paris, France; Department of Gynecology (C.L.), Centre Hospitalier Universitaire du Bocage, 21000 Dijon, France; Sanofi-Aventis Research Center (T.C.), 20137 Milan, Italy; Laboratory of Cardiovascular Physiopathology and Pharmacology (M.B.), Faculty of Medicine, 21079 Dijon, France*

**To assess whether pregnancy might influence the functionality and expression of human myometrial $\beta_2$- and $\beta_3$-adrenoceptors ($\beta_2$- and $\beta_3$-AR), we performed functional, binding, Western blot, and molecular biology experiments in human nonpregnant and near-term pregnant myometrium. Inhibition of spontaneous contractions induced by a $\beta_3$-AR agonist, SR 59119A, was significantly greater in pregnant, compared with nonpregnant, myometrial strips ($E'_{max}$ = 61 ± 5% *vs.* 44 ± 5% for pregnant and nonpregnant myometrium, respectively), whereas salbutamol, a $\beta_2$-AR agonist, was significantly less efficient in pregnant, compared with nonpregnant, myometrium ($E_{max}$ = 29 ± 4 *vs.* 54 ± 8%). Although two populations of binding sites corresponding to $\beta_2$- and $\beta_3$-AR were identified in both nonpregnant and pregnant myometrium, we found a clear predominance of the $\beta_3$-AR subtype. Moreover, $\beta_3$-AR binding sites were up-regulated 2-fold in myometrium at the end of pregnancy. Both $\beta_2$- and $\beta_3$-AR mRNA were expressed in human nonpregnant and pregnant myometrium. Contrary to $\beta_2$-AR, the expression of the $\beta_3$-AR transcripts and immunoreactive proteins was increased in pregnant, compared with nonpregnant, myometrium. Such compelling data suggest a predominant role for $\beta_3$-AR in the regulation of human myometrium contractility, especially at the end of pregnancy, which might have important consequences for the clinical management of preterm labor. (*J Clin Endocrinol Metab* 90: 1644–1650, 2005)**

THE INCIDENCE OF premature birth has risen over the past 15 yr, mainly because of medically induced births, yet spontaneous preterm delivery still remains stable and relatively high in developed countries despite preventative measures (1, 2). Approximately 6–12% of all pregnancies end up prematurely in Western countries, and deaths of premature babies represent approximately 85% of all perinatal mortality. Several stimulatory and inhibitory pathways regulate the balance of uterine quiescence and contractile activity during pregnancy. The mechanisms that govern the switch between these opposing functional states are still poorly understood. Worldwide, $\beta_2$-adrenoceptor (AR) agonists are the most commonly used tocolytic agents. However, besides their maternal and fetal side effects, their efficacy remains controversial (3, 4). $\beta_2$-ARs may vary according to hormonal status in human (5) or rat (6). The different proportion of each $\beta$-AR in pregnant and nonpregnant myometrium in goat and cow was suggested over 20 yr ago by Larsen (7). We previously demonstrated that $\beta_2$-ARs were predominant (~65%) over $\beta_1$-AR in the human pregnant myometrium and that the number of $\beta$-ARs was diminished at the end of pregnancy (8). An additional $\beta$-AR subtype, the $\beta_3$-AR, has since been characterized (9), and its functionality has been recently shown in the human near-term myometrium (10). Because adrenergic responsiveness is dependent on hormonal regulation (11, 12), we studied the status of the $\beta_3$-AR in comparison with the $\beta_2$-AR in the myometrium of near-term and nonpregnant women. More specifically, we investigated the functional contractile inhibition of specific $\beta_2$- and $\beta_3$-AR agonists, salbutamol and SR 59119A, respectively, concomitantly with the binding properties and the expression of the transcripts and the immunoreactive proteins of these receptors.

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Abbreviations: ADU, Arbitrary densitometric unit; AR, adrenoceptor; $B_{max}$, maximal binding capacity; CHO, Chinese hamster ovary; CRC, concentration-response curve; $E_{max}$, maximal effect; ICYP, (−)-[$^{125}$I]iodocyanopindolol; $K_D$, dissociation constant; Ki, inhibition constant; TBST, Tris-buffered saline/Tween 20.

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## Materials and Methods

### *Biological samples*

Myometrial biopsies were obtained from nonpregnant cycling women undergoing hysterectomy for benign gynecological indications or from pregnant women delivered by elective cesarean section before the onset of labor (38–40 wk of pregnancy) because of a diagnosed cephalopelvic disproportion in an otherwise uncomplicated normal pregnancy. Myometrial strips were excised from an immediately subserosal site in which the majority of the fibers are in a longitudinal orientation at an antiplacental site, as previously described (13). This study was approved by the Comité Consultatif de Protection des Per-

sonnes pour la Recherche Biomédicale (Paris-Cochin, France), and written informed consent was obtained from all donors.

### *Functional contractile study*

Segments of myometrium (8–12 × 2–3 mm) were suspended isometrically under a resting tension of 2 g in a 10-ml organ bath containing Krebs solution (composition, millimoles: NaCl, 118; KCl, 5.4; $CaCl_2$, 2.5; $KH_2PO_4$, 0.6; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 11.7; ascorbic acid, 0.1) maintained at 37 C and continuously exposed to a mixture of 95% oxygen-5% carbon dioxide (pH 7.4). The 2-g initial tension was chosen according to previous reports including our own experiments (10, 14). One end of each strip was connected to a force-displacement transducer, and tension changes were measured using Pioden strain gauges (UF1) that were amplified (EMKA, Paris, France) and recorded on a pen-writing oscillograph (Linseis, L65514, Munich, Germany). After 1 h, during which the myometrial strips were washed every 15 min and the resting tension readjusted to 2 g, the strips were then allowed to equilibrate for an additional hour until they showed regular spontaneous rhythmic contractile activity. Once contractions became regular in both amplitude and frequency, cumulative concentration-response curves (CRCs) (from 0.01 μM to 30 μM) were determined to characterize the effects of exposure to either SR 59119A ($\beta_3$-AR agonist, Sanofi-Aventis, Milan, Italy) or salbutamol ($\beta_2$-AR agonist, Sigma, St. Quentin Fallavier, France). The exposure time for each additional concentration was around 20–40 min, time necessary to reach a plateau of contractions. Because 3–4 h were necessary for the construction of a cumulative CRC, only one complete curve was obtained for each strip. Because we previously described that there was no time-related loss of contractions during our experiments (10, 13), we did not use time-matched control in this study. For salbutamol, because saturation of CRCs was reached, the maximal effect ($E_{max}$) was determined as a percentage of inhibition of initial amplitude of spontaneous contractions.

Potency was expressed as $pIC_{50}$, which is the $-\log IC_{50}$, where $IC_{50}$ is the concentration of agonist producing half of its maximal response. Because saturation of CRC was not reached for SR 59119A, $E'_{max}$ indicates the maximal response obtained at the maximal concentration tested (30 μM), and potency was not calculated. Data were analyzed by nonlinear regression sigmoidal dose-response curve using the Prism 4.01 software (GraphPad Software, San Diego, CA). The equation curve is $Y = Y_{min} + (Y_{max} - Y_{min})/[1 + 10\hat{}(\log IC_{50} - X)]$ where X is the logarithm of concentration, Y is the response, $Y_{min}$ is the minimal inhibitory effect, and $Y_{max}$, the maximal inhibitory effect.

### *Binding studies*

Crude membranes were prepared from different biopsy specimens of near-term pregnant or nonpregnant myometrial tissues as reported previously (8, 15). Membranes were diluted at a protein concentration of 1–3 $mg \cdot ml^{-1}$, in a cold 50 mM Tris-HCl (pH 7.4) buffer. Protein concentration was determined by the method of Bradford (16) using BSA as standard. Binding studies were performed at 30 C for 60 min in a 0.25-ml crude membrane preparation (12 μg protein per sample) that contained 50 mM Tris-HCl (pH 7.4), 154 mM NaCl, 0.1% BSA, and (−)-[$^{125}$I]iodocyanopindolol (ICYP) from 2 to 1000 pM (saturation analysis). The ICYP (specific activity 2200 Ci/mmol) was obtained from PerkinElmer Life Sciences (Boston, MA). Reactions were terminated by dilution with 5 ml of ice-cold 50 mM Tris-HCl (pH 7.4), 0.01% Triton X-100, and filtration over glass-fiber filters (Whatman GF/C, Maidston, UK) presoaked in 0.1% BSA in 50 mM Tris-HCl (pH 7.4). The filters were washed with an additional 15 ml of 50 mM Tris-HCl (pH 7.4) and 0.01% X-100 Triton, dried, and counted on a Packard γ-counter with 85% efficiency.

In previous studies examining $\beta_2$-AR (8), specific binding was defined as the difference between the amount of ICYP bound in the absence (total binding) and presence (nonspecific binding) of 500 μM unlabeled (−)-isoproterenol (Sigma). For $\beta_3$-AR binding studies, nonspecific binding was defined by the amount of ICYP bound in the presence of unlabeled (±)-alprenolol (500 μM, Sigma). Competition experiments were carried out at a fixed concentration of ICYP (400 pM) with increasing concentrations of competing agents and in the presence of 0.1 μM (−)-propranolol to block $\beta_1$- and $\beta_2$-AR (17). At ligand concentrations near the ICYP dissociation constant ($K_D$), nonspecific binding was less than 15 and 30% of total binding for $\beta_2$- and $\beta_3$-AR, respectively. The inhibition constants values (Ki) were calculated by the equation of Cheng and Prusoff (18): $Ki = IC_{50}/(1 + [L]/K_D)$, where $IC_{50}$ is the concentration of competing agent producing 50% of a maximal response, [L] is the concentration of ICYP used in the assay, and the appropriate $K_D$ value is the dissociation constant of ICYP as determined by nonlinear regression. Radioligand binding data were analyzed by nonlinear regression using the GraphPad Prism 4.01 software. Results are expressed as SEM.

### *RT and PCR analysis*

As described previously (15, 19), extraction of total RNA from myometrial tissues using the Trizol reagent method, reverse transcription using Muloney murine leukemia virus reverse transcriptase, and PCR using *Taq* DNA polymerase were performed under the conditions recommended by the manufacturer (Life Technologies, Cergy Pontoise, France). The primers were designed according to Roberts *et al.* (20). The amplification profile for $\beta_2$- and $\beta_3$-AR consisted in denaturation at 94 C for 1 min, annealing at 64 C for 1 min, extension at 72 C for 1 min with a final extension at 72 C for 10 min. An aliquot from the PCR mixture was resolved by electrophoresis on a 2% agarose gel (Life Technologies) and visualized by ethidium bromide staining under UV light. To check the size of PCR products, a DNA molecular mass standard ladder (123-bp DNA ladder, Life Technologies) was concomitantly subjected to electrophoresis. An additional control of validity was carried out using Southern blot analysis of the PCR products with specific internal oligonucleotides (data not shown). Amplification of an endogenous marker, the human $\beta_2$-microglobulin cDNA (21), was performed as internal control. Absence of DNA contamination was confirmed by conducting PCR control reactions containing mRNA without reverse transcriptase. The intensities of the bands on Polaroid pictures of the ethidium bromide staining gel were analyzed densitometrically using the NIH Image 1.62 program (National Institutes of Health, Bethesda, MD). Results are expressed as relative levels [arbitrary densitometric units (ADUs)] of specific mRNA normalized to $\beta_2$-microglobulin mRNA in each sample.

### *Western blotting analysis*

Snap-frozen myometrial tissues obtained from nonpregnant and near-term pregnant women were homogenized with Ultra-Turrax in homogenization buffer [10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 40 $mg \cdot ml^{-1}$ leupeptine, 2 mM Pefabloc]. After an initial centrifugation at 500 × *g* for 15 min at 4 C, the supernatant was removed and centrifuged at 48,000 × *g* for 20 min at 4 C. The resulting pellet was suspended in solubilization buffer (300 mM NaCl, 50 mM $Na_2HPO_4$, 1% Triton X-100) overnight at 4 C. A further centrifugation was performed at 48,000 × *g* for 20 min at 4 C. Total protein content was determined by the Bradford method with BSA as standard. Samples (40 μg protein by lane) were dissolved (vol/vol) in 2× Laemmli buffer (22) and boiled for 5 min before electrophoresis on a 10% SDS-PAGE. Proteins were transferred to a nitrocellulose membrane (Hybond-P, Amersham Biosciences, Freiburg, Germany). To block nonspecific antibody binding, blots were incubated for 1 h in 10% nonfat dried milk powder in Tris-buffered saline/Tween 20 (TBST) [10 mM Tris, 150 mM NaCl, and 0.1% Tween 20 (pH 7.8)] at room temperature. Blocked membranes were washed three times with TBST. The blots were then incubated overnight at 4 C with a 1:500 dilution of primary $\beta_2$-AR polyclonal antibody (H-20, sc-569, Santa Cruz Biotechnology, Santa Cruz, CA) or a 1:500 dilution of primary $\beta_3$-AR polyclonal antibody (AB5122, Chemicon International, Temecula, CA) in 1% nonfat dried milk powder in TBST. After three washes with TBST, the blots were incubated for 45 min with horseradish peroxidase-conjugated antirabbit IgG whole antibody (NA 934, Amersham) at a dilution of 1:5000 at room temperature and washed five times with TBST. Immunoreactive proteins were detected by chemiluminescence (ECL detection reagents, RPN2105, Amersham Pharmacia Biotech, Little Chalfont, UK). The intensities of the bands were analyzed densitometrically using the NIH Image 1.62 program. Results are expressed as the mean ± SEM in ADUs. The specificity of each immunoreactive band was assessed by specific blocking in the presence of the respective antigenic peptide against which the antibodies have been raised (sc-569P from Santa Cruz Biotechnology for $\beta_2$-AR and AG388 from Chemicon International for $\beta_3$-AR).

For preadsorption, a mixture of each primary antibody with its re-

spective antigen (1:5) was incubated under mild agitation in a small volume of PBS buffer for 2 h at room temperature (for $\beta_2$-AR) or 24 h at 4 C (for $\beta_3$-AR). In addition, homogenate of Chinese hamster ovary (CHO) cells transfected with the human $\beta_3$-AR (graciously provided by Dr. P. Marini, Sanofi-Aventis Research Center, Milan, Italy) was used as positive control.

### *Drug and solution*

SR 59119A (*N*-[(7-methoxy-1,2,3,4-tetrahydronaphtalen-(2R)-2-yl)-methyl]-(2R)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride) was a gift from Sanofi-Aventis, Sanofi-Aventis Research Center. SR 59119A was dissolved in a mixture of absolute ethanol 30%, dimethylsulfoxide 2%, and distilled water for the 30-$\mu$M solution and thereafter diluted in distilled water. Salbutamol sulfate was dissolved in distilled water. In the functional studies, drug concentrations were given as final bath concentrations.

### *Statistical analysis*

In the functional experiments, differences among groups were determined by ANOVA and expressed with a *t* test using the Bonferroni correction. Differences in potencies and maximal effects, as well as differences among groups in binding experiments, were determined by Student's *t* test for unpaired data or ANOVA as appropriated. All differences were considered significant when $P < 0.05$.

## Results

### *Functional contractile study*

Application to muscle strips of SR 59119A ranging from 0.1 to 30 $\mu$M caused a concentration-dependent inhibition of the spontaneous contractile activity of myometrium obtained from nonpregnant or near-term women (Fig. 1). A comparison of the CRC for both $\beta_2$- and $\beta_3$-AR agonists in pregnant and nonpregnant myometrium is shown in Fig. 2, A and B. Application to muscle strips of salbutamol or SR 59119A, ranging from 0.01 to 30 $\mu$M, caused a concentration-dependent inhibition of the spontaneous contractile activity of myometrial strips obtained from nonpregnant or near-term pregnant women. In the near-term myometrium, salbutamol effect was weaker and the CRC was downward shifted, compared with that obtained in the nonpregnant myometrium (Fig. 2A, ANOVA, $P < 0.001$). The $E_{max}$ value of salbutamol was significantly ($P < 0.05$) lower in the pregnant than in the nonpregnant tissue ($E_{max} = 29 \pm 4$ and $54 \pm 8\%$ for pregnant and nonpregnant myometrium, respectively), whereas no significant difference was observed between the $pIC_{50}$ values (Table 1). SR 59119A was responsible for a strong inhibition of spontaneous contractions in both the pregnant and nonpregnant myometrium (Fig. 2B). In contrast to salbutamol, the CRC of SR 59119A obtained in the near-term myometrium was shifted to the left, compared with that obtained in the nonpregnant myometrium (Fig. 2B, ANOVA, $P < 0.01$). This was associated with an increase, although not statistically significant, in $E'_{max}$ values ($61 \pm 5$ *vs.* $44 \pm 5\%$ for pregnant and nonpregnant myometrium, respectively) (Table 1). In pregnant myometrium, the CRC of salbutamol was shifted to the right, compared with that of SR 59119A (ANOVA, $P < 0.01$), and the effect obtained at the maximal dose tested (30 $\mu$M) was lower for salbutamol than SR 59119A ($29 \pm 4$ *vs.* $61 \pm 5\%$ for salbutamol and SR 59119A, respectively, $P < 0.001$) (Table 1).

### *Characterization of $\beta_2$- and $\beta_3$-AR binding sites*

ICYP bound to membrane preparations of nonpregnant and pregnant myometrium in a concentration-dependent manner. Scatchard representation of saturation curve is shown in Fig. 3A. In pregnant myometrium, nonlinear analysis of the saturation curve demonstrated the appearance of a two-site plot, indicating both a low-affinity site [$K_D = 771 \pm 109$ pM; maximal binding capacity ($B_{max}$) = $92.9 \pm 13.1$ fmol·mg$^{-1}$ protein] and a high-affinity site ($K_D = 39 \pm 4$ pM; $B_{max} = 4.9 \pm 0.6$ fmol·mg$^{-1}$ of protein) (Table 2). Consistent

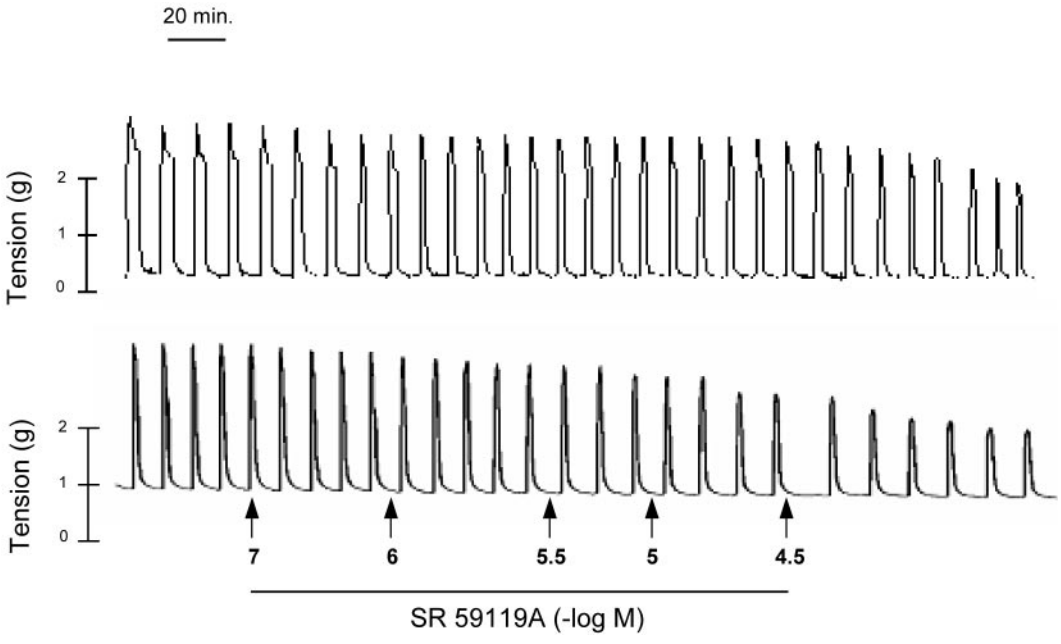


FIG. 1. Representative recording of the effect of SR 59119A on spontaneous contractions of human nonpregnant (*upper trace*) and near-term (*lower trace*) myometrial strips.

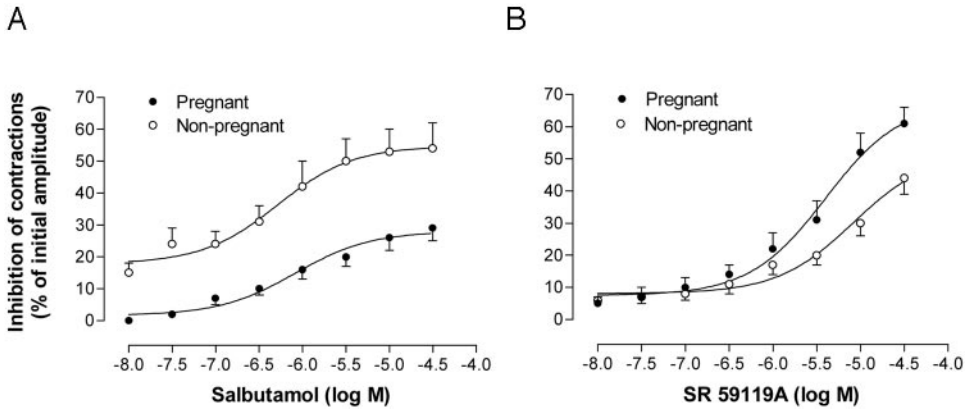


FIG. 2. Effect of salbutamol ($\beta_2$-AR agonist, A) or SR 59119A ($\beta_3$-AR agonist, B) on spontaneous contractions of myometrium from nonpregnant (*white circles*) or pregnant (*black circles*) women. Results are expressed as mean ± SEM (n = 11–31).

with our previous results and those from other groups working on human pregnant myometrium (8, 23), the high-affinity binding site found here resembled that of a $\beta_2$-AR profile. The identity of the low-affinity binding site was confirmed by competition binding experiments with the selective $\beta_3$-AR agonist. SR 59119A competed at the propranolol-resistant ICYP binding site (Fig. 3B) with a Ki value of 276 ± 76 nM. Competition binding data were similar (Ki = 154 ± 82 nM) in nonpregnant myometrium (data not shown). It should be noted that the slope value for these curves was low (Hill coefficient nH = 0.71 and 0.65 in nonpregnant and pregnant myometrium, respectively), indicating that two sites may be present but cannot be significantly differentiated by computer analysis. Nonlinear analysis of saturation curves revealed that the $K_D$ values of $\beta_3$-AR in the nonpregnant and pregnant myometrium were significantly ($P < 0.005$) different (Fig. 3A and Table 2). In addition, the $B_{max}$ of $\beta_3$-AR was significantly ($P < 0.01$) greater in pregnant than nonpregnant myometrium (Fig. 3A and Table 2). For $\beta_2$-AR, the $K_D$ values between nonpregnant and pregnant myometrium were not significantly different, just as were the $B_{max}$ values (Table 2).

*Expression of $\beta_2$- and $\beta_3$-AR transcripts*

As illustrated in Fig. 4A, electrophoresis on agarose gel stained with ethidium bromide revealed PCR products of the correct predicted sizes, 417 bp for $\beta_2$-AR and 368 bp for $\beta_3$-AR, in both pregnant and nonpregnant myometrial samples. We confirmed successful normalization of RNA amounts by obtaining equivalent intensity for the $\beta_2$-microglobulin band in pregnant and nonpregnant tissues. Densitometric analysis of the gels indicated a slight decrease in the signal for $\beta_2$-AR mRNA (−27.4%) in near-term myometrium comparatively with nonpregnant tissue, whereas the signal for $\beta_3$-AR mRNA was increased in pregnant myometrium (+66.3%) (Fig. 4B).

**TABLE 1.** Maximal effect ($E_{max}$ and $E'_{max}$) and potency ($pIC_{50}$) values for salbutamol and SR 59119A on spontaneous contractile activity of myometrium from nonpregnant and near-term pregnant women

| | Salbutamol ($\beta_2$-AR agonist) | | | SR 59119A ($\beta_3$-AR agonist) | | |
|---|---|---|---|---|---|---|
| | n | $E_{max}$ | $pIC_{50}$ | n | $E'_{max}$ | $pIC_{50}$ |
| Nonpregnant | 11 | 54 ± 8% | 6.74 ± 0.15 | 12 | 44 ± 5% | ND |
| Pregnant | 31 | 29 ± 4%[a] | 6.11 ± 0.11 | 24 | 61 ± 5% | ND |

n, Number of experiments; ND, not determined. Each experiment was conducted on tissue from a different patient.
[a] $P < 0.05$ *vs.* nonpregnant.

*Expression of $\beta_2$- and $\beta_3$-AR immunoreactive proteins*

Western blotting of plasma membranes prepared from nonpregnant and pregnant myometrium revealed a 67-kDa band corresponding to $\beta_2$-AR (24) (Fig. 5A). For $\beta_3$-AR, the Western blotting revealed a main 68-kDa band (Fig. 5A), which disappears in the presence of the corresponding blocking peptide (data not shown). As positive control, a 68-kDa band was observed with a homogenate of CHO cells transfected with the human $\beta_3$-AR that was also specifically blocked by the antigenic peptide (data not shown). Densitometric immunoblot analysis indicated no change in the intensity of the signal for the $\beta_2$-AR in the myometrium of near-term, compared with nonpregnant, women. At the opposite, an increase (+40%) in the intensity of the signal for the $\beta_3$-AR protein was observed in pregnant, compared with nonpregnant, myometrium (Fig. 5B).

## Discussion

A few years ago, we first described the presence of a functional $\beta_3$-AR positively coupled to cAMP production in human near-term myometrium (10). We recently demonstrated that contrary to $\beta_2$-ARs, $\beta_3$-ARs are less prone to sustained agonist-induced desensitization in near-term myometrium (15). The present study strengthens these previous reports by providing the first evidence not only that $\beta_3$-ARs are present but also that they are the predominant subtype, compared with the $\beta_2$-ARs, both in human nonpregnant and pregnant myometrium (10- and 20-fold more numerous, respectively). Furthermore, our results indicate that human $\beta_3$-ARs are up-regulated in pregnancy.

These new findings appear to contradict the conventional belief that in human (8, 23) and other species (25, 26), $\beta_2$- and $\beta_1$-ARs account, respectively, for approximately 65 and 35% of the entire myometrial β-AR population. $\beta_3$-ARs have not previously been detected, probably due to their low affinity for the ICYP ligand commonly used for β-AR binding studies. Complementary approaches combining functional, binding, Western blotting, and molecular biology experiments were carried out to fully characterize the changes that might occur regarding myometrial β-AR status at the end of pregnancy. Our functional

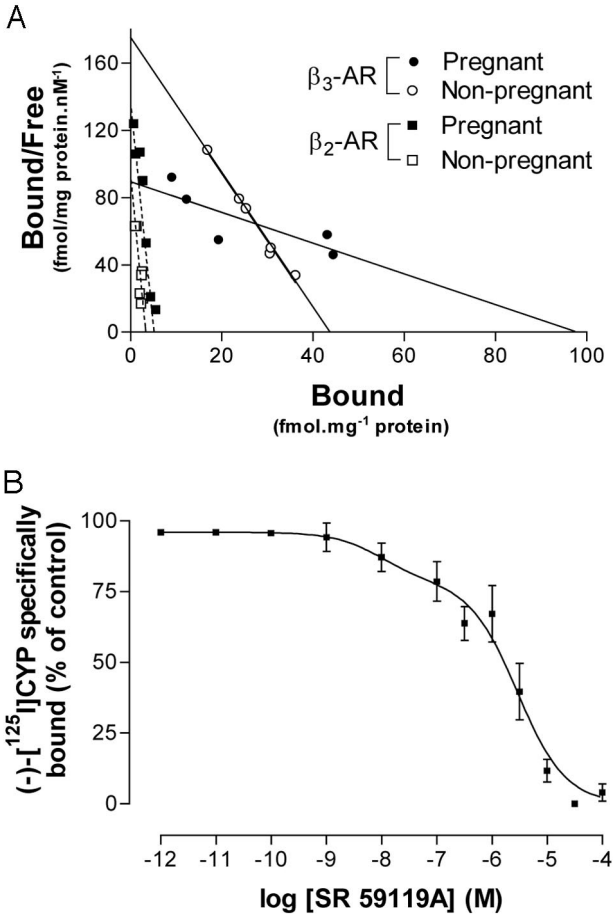


FIG. 3. A, Representative Scatchard saturation plot of total binding of ICYP to human nonpregnant and pregnant myometrial membranes. Although linear Scatchard plots are represented in this figure, a nonlinear regression method was used to determine the $K_D$ and $B_{max}$ values. B, Competition between ICYP and SR 59119A ($\beta_3$-AR agonist) for binding sites in membrane preparations of myometrium from pregnant women. Results are expressed as mean ± SEM from eight different pregnant women.

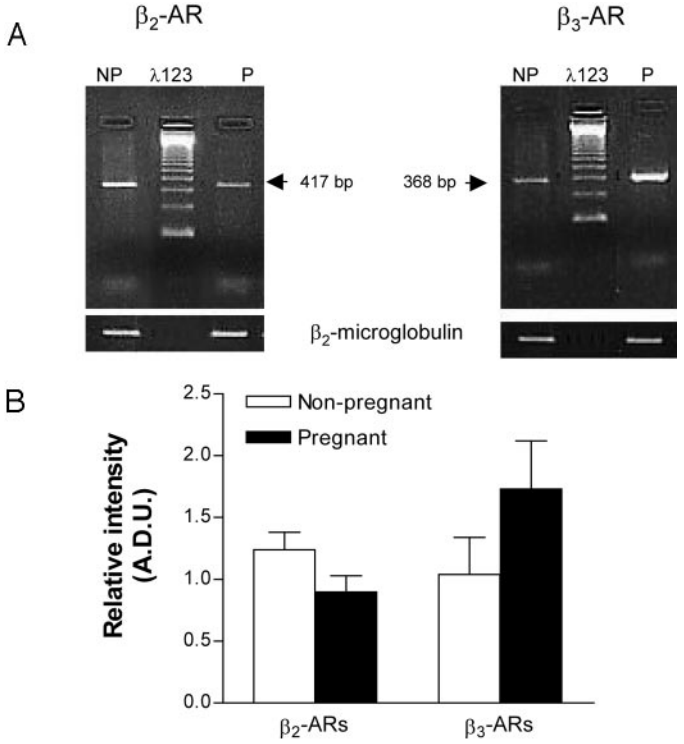


FIG. 4. A, Representative RT-PCR analysis of the mRNA steady-state levels for $\beta_2$- and $\beta_3$-AR in human nonpregnant (NP) and pregnant (P) myometrium. The 123-bp DNA ladder (λ123) was used to estimate the size of the PCR products, and $\beta_2$-microglobulin expression was used as a standard reference. These experiments were performed on myometrium from six pregnant and six nonpregnant women. B, Densitometric analysis of the relative expression of $\beta_2$- and $\beta_3$-AR transcripts in myometrium of nonpregnant (NP) and pregnant (P) women. A.D.U. represents the ratio between the intensity of the PCR fragments for $\beta_2$- or $\beta_3$-AR and the intensity of the signal for the internal standard $\beta_2$-microglobulin. Each *bar* represents the mean ± SEM from six different women.

experiments demonstrated that the selective $\beta_3$-AR agonist, SR 59119A, was a more efficient inhibitor of spontaneous contractions in pregnant than nonpregnant myometrium. The $\beta_2$-AR agonist salbutamol, in contrast, displayed a weaker inhibition of spontaneous contractions in the near-term, compared with the nonpregnant, myometrium. Few studies have been performed to compare nonpregnant and pregnant myometrial tissues. In 1979, Larsen (7) reported that isoproterenol, a nonselective β-AR agonist, and salbutamol and ritodrine, both selective $\beta_2$-AR agonists, caused a concentration-dependent relaxation of pregnant goat and cow myometrium, whereas only isoproterenol was able to reduce the amplitude of spontaneous contractions of nonpregnant myometrium from the same animals.

**TABLE 2.** Affinity ($K_D$) and number of $\beta_2$- and $\beta_3$-AR binding sites ($B_{max}$) determined after saturation binding experiments in human nonpregnant and pregnant myometrium

| | $\beta_2$-AR | | $\beta_3$-AR | |
|---|---|---|---|---|
| | Nonpregnant | Near-term | Nonpregnant | Near-term |
| n | 3 | 4 | 6 | 8 |
| $K_D$ (pM) | 78 ± 28 | 39 ± 4 | 218 ± 66 | 771 ± 109[a] |
| $B_{max}$ (fmol·mg$^{-1}$ of protein) | 4.4 ± 1.2 | 4.9 ± 0.6 | 40.0 ± 4.9 | 92.9 ± 13.1[b] |

n, Number of patients.
[a] $P < 0.005$ *vs.* nonpregnant myometrium.
[b] $P < 0.01$ *vs.* nonpregnant myometrium.

Binding competition studies, transcripts, and protein analysis were performed concurrently to determine whether any changes in the mRNA and protein expression and the number of $\beta_2$- and $\beta_3$-AR binding sites might explain the observed change in the contractile response of nonpregnant and pregnant myometrium to $\beta_2$- and $\beta_3$-AR agonists. According to the work of Gsell *et al.* (27), we found no modification in the number of $\beta_2$-AR binding sites in nonpregnant, compared with pregnant, human myometrium. We confirmed these results by Western blotting because, in agreement with Chanrachakul *et al.* (24), we did not find any convincing difference in the level of expression of $\beta_2$-AR immunoreactive protein between human nonpregnant and near-term myometrium. Analysis of the level of transcripts revealed a slight decrease in $\beta_2$-AR mRNA in pregnant, compared with nonpregnant, women. This discrepancy between changes in mRNA expression and the number of binding

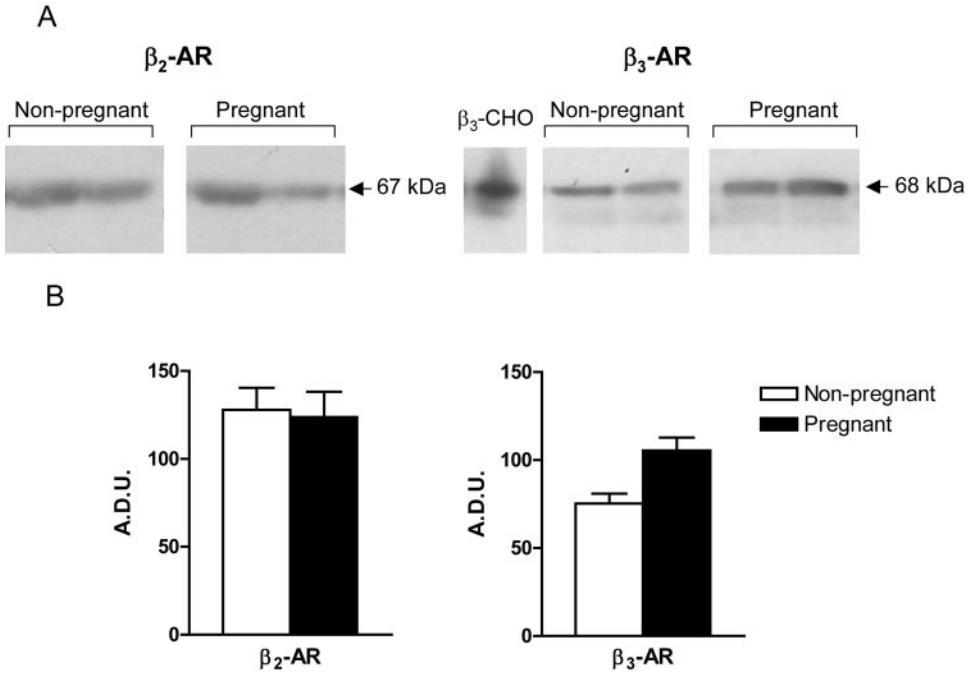


FIG. 5. A, Representative Western blot analysis of $\beta_2$- and $\beta_3$-AR expression in plasma membranes from myometrium of nonpregnant and pregnant women. Expected sizes are 67 kDa for $\beta_2$-AR and 68 kDa for $\beta_3$-AR. These experiments were performed on myometrium from five nonpregnant and five pregnant women. Homogenate of CHO cells transfected with the human $\beta_3$-AR ($\beta_3$-CHO) was used as positive control. B, Analysis of the expression of $\beta_2$- and $\beta_3$-AR immunoreactive proteins in myometrium of nonpregnant and pregnant women. A.D.U. represents the intensity of the bands evaluated by densitometry. Each *bar* represents the mean ± SEM from five different nonpregnant and five different pregnant women.

sites has already been described (28), and might be explained by an increased stability of mRNA, an enhanced translation rate, or a low receptor turnover (29, 30). The difference in the relaxant effect of salbutamol observed between nonpregnant and pregnant myometrium cannot be completely cleared up by our molecular and biochemical results, and we speculate that changes in coupling between receptor and adenylyl cyclase in both tissues are likely to explain these data.

Contrary to what was observed for $\beta_2$-ARs, the increase in the level of expression of $\beta_3$-AR transcripts in near-term, compared with nonpregnant, myometrium was associated with an increase of the signal for the corresponding immunoreactive protein and accompanied by an increase in the number of $\beta_3$-AR binding sites. Based on the differences in affinity for ICYP, we established that human myometrial $\beta_3$-ARs have a lower affinity than $\beta_2$-ARs. These results are in accordance with the atypically low affinity and potency of $\beta_3$-ARs for conventional β-AR antagonists (31). Such low affinity of $\beta_3$-ARs for ICYP was also found in another smooth muscle, the internal anal sphincter (32), a tissue in which the $\beta_3$-AR is predominant over the $\beta_1/\beta_2$-AR subtypes. The SR 59119A effect appears to be selective of a $\beta_3$-AR stimulation and not linked to a nonselective binding on $\beta_2$-ARs. Indeed, we have previously shown that SR 59119A-induced inhibition of human colon (33) or near-term myometrium (10) spontaneous contractions was unaffected by the blockade of $\beta_1$- and $\beta_2$-ARs by propranolol (0.1 μM) but was significantly reduced by the selective $\beta_3$-AR antagonist SR 59230A (1 μM). Furthermore, functional studies on human isolated bronchi, a tissue that expresses predominantly the $\beta_2$-AR subtype, have shown that SR 59119A was devoid of any $\beta_2$-AR agonist (33) or antagonist (34) properties.

Our study confirms, as previously shown in either human (8) or rat (35) that pregnancy might influence β-AR expression. The influence of sexual hormones on the expression of myometrial $\beta_2$-AR is well documented. Progesterone may increase the density of $\beta_2$-AR in late-pregnant rats (12) by controlling the transcription rate of the gene (11). In guinea pig, estradiol treatment resulted in a marked up-regulation of $\beta_2$-AR density, whereas progesterone influenced $\beta_2$-AR to a lesser degree (36). By contrast, little is known about modulation of $\beta_3$-ARs by steroid hormones. Pregnancy in the human is associated with a progressive rise in the myometrial 17β-estradiol to progesterone ratio (37), and it is of interest to note that an increase in the myometrial response to a $\beta_3$-AR agonist was observed near term when this hormonal ratio is the most elevated, compared with the nonpregnant myometrium. Recent results on brown adipocytes demonstrated that estradiol and progesterone are able to modify $\beta_3$-AR affinity and density (38). These data allow us to hypothesize that hormonal changes observed during pregnancy are likely to explain the modulation of $\beta_2$- and $\beta_3$-AR expression, functionality, and affinity observed in pregnant, compared with nonpregnant, myometrium. However, such a hypothesis remains to be tested with further experiments.

One of the major limitations for $\beta_2$-AR agonists' use in clinical practice is their maternal and fetal cardiovascular side effects. The presence and function of $\beta_3$-AR in the cardiovascular system are still a matter of debate (39, 40). However, clinical trials regarding the effect of $\beta_3$-AR agonists on human metabolism did not reveal any change in heart rate, blood pressure, plasma glucose, insulin, or potassium levels (41–43). Another limiting factor for the use of selective $\beta_2$-AR agonists as tocolytic drugs is that their effect, if any, appears to be of relatively short duration (44). This loss of efficacy is due in part to desensitization of their receptors. A distinguishing feature of the $\beta_3$-AR, compared with $\beta_2$-AR, subtype is its relative lack of desensitization either *in vitro* or *in vivo* after activation with agonists (15, 45).

In conclusion, the present study provides compelling evidence for the predominance of the $\beta_3$-AR subtype in the human myometrium and its up-regulation in pregnancy. In light of these findings and our recent data on their resistance

to desensitization (15), we suggest that $\beta_3$-AR agonists may have considerable future pharmaceutical implications in the clinical management of preterm labor.

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Address all correspondence and requests for reprints to: Céline Rouget, Unité Propre de Recherche de l'Enseignement Supérieur EA220–Pharmacology, Unité de Formation et de Recherche Biomédicale des Saints Pères, 45 rue des Saints Pères, F-75006 Paris, France. E-mail: celine.rouget@univ-paris5.fr.

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